



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF
CLAUSENA EXCAVATA AND SOME CITRUS SPECIES (RUTACEAE)**

PEH TIAN HAI

FSAS 2001 31

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF
CLAUSENA EXCAVATA AND SOME CITRUS SPECIES (RUTACEAE)**

By

PEH TIAN HAI

**Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of
Science in the Faculty of Science and Environmental Studies
Universiti Putra Malaysia**

January 2001



Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirement for the degree of Master of Science.

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF
CLAUSENA EXCAVATA AND SOME CITRUS SPECIES (RUTACEAE)**

By

PEH TIAN HAI

January 2001

Chairman : Dr. Taufiq Yap Yun Hin

Faculty : Science and Enviromental Studies

Investigations on the leaves and stem bark of *Clausena excavata* (Rutaceae) have resulted in the isolation of twelve compounds. The structures of these compounds were elucidated by using spectroscopic techniques such as NMR, MS, IR and UV and also by comparison with previous reports.

The stem bark of *C. excavata* collected from Jabi, Kedah yielded two new carbazole alkaloids, 1,8-dihydroxy-3-formyl-4-prenylcarbazole and 3-carbomethoxy-2-hydroxy-7-methoxycarbazole, three known carbazole alkaloids, clausine-H, clausine-K and clausine-B together with one coumarin, scopoletin, two limonoids (clausenarin and CEA 10), and one triterpene, stigmasterol. From the leaves of *C. excavata*, a known carbazole alkaloid, 3-formyl-2,7-dimethoxycarbazole together with β -sitosterol and safrole were isolated.

The essential oils were obtained by hydrodistillation using fresh leaves and analysed using GC-MS spectrometry. The main essential oil components from *Clausena excavata* (Jabi A) were saffrole and α -terpinolene, while Jabi B gave saffrole and terpinolene as major constituents. The essential oils from several species of Citrus of Malaysia have also been investigated. Citronellal and citronellol were the major components of *C. hystrix*. The major constituents of *C. aurantifolia* were limonene, (z)-citral and (e)-citral. The major components of the oils of *C. sinensis* were found to be linalool and β -elemene. Germacrene-D, elemol and β -eudesmol were the major constituents of the oil of *C. microcarpa*. The oil of *C. maxima* (Kedah) contained significant amounts of sabinene, γ -terpinene and β -caryophyllene. However, β -phellandrene, *trans*- β -ocimene and β -caryophyllene are major constituents of *C. maxima* (Johor).

1,8-dihydroxy-3-formyl-4-prenylcarbazole, 3-carbomethoxy-2-hydroxy-7-methoxycarbazole and Clausine-K gave very strong activities against CEM-SS cells line with IC₅₀ of 2.1, 8.2 and 5.1 μ g/ml, respectively. However, crude hexane and EA extracts from the stem bark of *C. excavata* gave significant cytotoxic effect with IC₅₀ of 3.27 and 2.47 μ g/ml, respectively. However, All essential oils gave significant cytotoxic activity with IC₅₀ value around 3 μ g/ml.

The antimicrobial activity test against four types of bacteria: *Bacillus subtilis* mutant, *Bacillus subtilis* wild type, *Staphylococcus aureus* and *Pseudomonas aeruginosa* also carried out on crude extracts and essential oils. All the stem bark crude extracts of *C. excavata* and essential oil of *Citrus microcarpa* showed active against all the bacteria used.

The susceptibility or resistance of mosquito larvae of *Aedes aegypti* to insecticide test on crude extracts of *C. excavata* revealed that the hexane extracts of the stem bark, leaves and EA extract of leaves were strongly cytotoxic to the mosquito larvae. All the essential oils showed very strong cytotoxicity to the mosquito larvae with an extremely low LC₅₀ value less than 50 µg/ml.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Master Sains.

KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA *CLAUSENA EXCAVATA* DAN BEBERAPA SPESIES CITRUS (RUTACEAE)

Oleh

PEH TIAN HAI

Januari 2001

Pengerusi : Dr. Taufiq Yap Yun Hin

Fakulti : Sains dan Pengajian Alam Sekitar

Kajian ke atas ekstrak mentah daun dan kulit batang *Clausena excavata* (Rutaceae) telah menghasilkan dua belas komponen. Struktur sebatian-sebatian ini telah dapat dikenalpasti dengan menggunakan kaedah spektroskopi seperti NMR, MS, IR dan UV dan juga perbandingan dengan kajian-kajian yang lepas.

Pengekstrakan dan pemencilan daripada kulit batang *C. excavata* telah menghasilkan dua alkaloid karbazole baru, 1,8-dihidroksi-3-formil-4-prenilkarbazole dan 3-karbometoksi-2-hidroksi-7-metoksikarbazole, dan tiga alkaloid karbazole yang telah dikenali, iaitu clausine-H, clausine-K dan clausine-B, satu koumarin, scopoletin, dua limonoid (clausenarin dan CEA 10), dan satu triterpene, stigmasterol. Daripada daun *C. excavata* pula, satu alkaloid karbazole yang telah dikenali, iaitu 3-formil-2,7-dimetoksikarbazole bersama dengan β -sitosterol dan safrole yang telah dipisahkan.

Minyak pati diperolehi dengan teknik penyulingan hidro yang menggunakan daun segar dan dianalisis dengan menggunakan kaedah spektrometri GC-MS. Komponen utama minyak pati daripada *Clausena excavata* (Jabi A) ialah safrole dan α -terpinolene. Manakala Jabi B pula mengandungi safrole dan terpinolene sebagai komponen utamanya. Kajian turut dilakukan ke atas minyak pati bagi beberapa spesies Citrus yang didapati di Malaysia. Citronellal and citronellol ialah komponen major bagi *C. hystrix*. Kandungan utama bagi *C. aurantifolia* adalah limonene, (z)-citral dan (e)-citral. Minyak pati daripada *C. sinensis* didapati major dalam linalool dan β -elemene. Germacrene-D, elemol dan β -eudesmol adalah komponen utama bagi minyak *C. microcarpa*. Minyak pati daripada *C. maxima* (Kedah) mengandungi amaun yang signifikan, iaitu sabinene, γ -terpinene dan β -caryophyllene. Manakala, β -phellandrene, *trans*- β -ocimene dan β -caryophyllene adalah komponen major bagi *C. maxima* (Johor).

1,8-dihidroksi-3-formil-4-prenilkarbazole, 3-karbometoksi-2-hidroksi-7-metokikarbazole dan clausine-K menunjukkan sangat aktif terhadap garisan sel CEM-SS dengan nilai $IC_{50} = 2.1, 8.2$ dan $5.1 \mu\text{g/ml}$ masing-masing. Manakala ekstrak mentah daripada kulit batang *C. excavata* memberi kesan sitotoksik yang signifikan terutamanya ekstrak mentah heksana dan EA ($IC_{50} = 3.27$ dan $2.47 \mu\text{g/ml}$ masing-masing). Manakala, Semua minyak pati memberi aktiviti sitotoksik yang signifikan di mana nilai IC_{50} di antara sekitar $3 \mu\text{g/ml}$.

Aktiviti antimikrobial terhadap empat jenis bakteria: *Bacillus subtilis* mutan, *Bacillus subtilis* jenis liar, *Staphylococcus aureus* yang tahan metisillin dan *Pseudomonas aeruginosa* juga telah dijalankan ke atas ekstrak mentah dan minyak

pati. Semua ekstrak mentah daripada kulit batang *C. excavata* dan minyak pati daripada *Citrus microcarpa* memberi aktiviti terhadap semua bakteria yang digunakan.

Pengaruh atau penentangan larva nyamuk *Aedes aegypti* kepada ujian insektiside terhadap ekstrak heksana daripada kulit batang dan daun *C. excavata* serta ekstrak EA daripada daun menunjukkan sitotoksiksiti yang tinggi terhadap larva nyamuk. Semua minyak pati yang diuji telah menunjukkan sitotoksiksiti yang tinggi ke atas nyamuk *Aedes aegypti* dengan nilai LC_{50} yang tersangat rendah, iaitu kurang daripada 50 $\mu\text{g/ml}$.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation and gratitude to my supervisor, Dr. Taufiq Yap Yun Hin for his continuous supervision, invaluable advice, guidance and valuable comments throughout the course of this project. I also wish to thank my supervising committee members Dr. Gwendoline Ee Cheng Lian, Prof. Dr. Mawardi Rahmani, Assoc. Prof. Dr. Abdul Manaf Ali and Assoc. Prof. Dr. Radzali Muse for their constant support.

My sincere thanks and deepest gratitude are also extended to Assoc. Prof. Dr. Mohd Aspollah Hj. Sukari for his financial support and encouragement. I am also very grateful to my colleagues, friends and staff of the Chemistry Department of UPM, Mr. Zainal Zahari Zakaria, Mr. Nordin Ismail, Mr. Zainuddin Samadi, Mrs. Rusnani Amirudin, Mrs. Ratna, Miss Ng Kim Nee, Mr. Hassan and Mr. Sugeng whose help, advice and encouragement were invaluable during this research project. Thanks Mr. Ramlan for plants collection.

My special thanks also goes to: Yih Yih, Boon Keat and Maizura from Biotechnology Department for their help on bioassay work involving cytotoxicity and antimicrobial test and Dr. Rusea Go for identifying the plant materials.

I also wish to thank my mother, brother, sisters and Sze Yuen for their patience, tolerance and moral support. Financial assistance from the Malaysian Government under the IRPA programme is gratefully acknowledged.

I certify that an Examination Committee met on 17th January 2001 to conduct the final examination of Peh Tian Hai on his Master of Science thesis entitled "Chemical Constituents and Biological Activities of *Clausena excavata* and Some Citrus Species (Rutaceae)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

MOHD ASPOLLAH HJ. SUKARI, Ph.D.

Associate Professor
Department of Chemistry
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

TAUFIQ YAP YUN HIN, Ph.D.

Lecturer
Department of Chemistry
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

GWENDOLINE EE CHENG LIAN, Ph.D.

Lecturer
Department of Chemistry
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

MAWARDI RAHMANI, Ph.D.

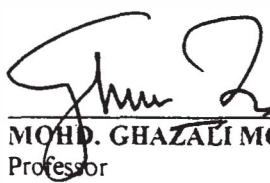
Professor
Department of Chemistry
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

ABDUL MANAF ALI, Ph.D.

Associate Professor
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

RADZALI MUSE, Ph.D.

Associate Professor
Department of Biochemistry and Microbiology
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)



MOHD. GHAZALI MOHAYIDIN, Ph.D.

Professor
Deputy Dean of Graduate School
Universiti Putra Malaysia

Date: 09 FEB 2001

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.



MOHD. GHAZALI MOHAYIDIN, Ph.D.

Professor

Deputy Dean of Graduate School

Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.



PEH TIAN HAI

Date: 09.02.2001

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL SHEETS	ix
DECLARATION FORM	xi
LIST OF TABLES	xiv
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xxi
 CHAPTER	
 1 INTRODUCTION	 1
1.1 Extraction and Isolation of Chemical Constituents from <i>Clausena excavata</i>	1
1.1.1 <i>Clausena excavata</i> species.....	1
1.2 Essential Oils from the Leaves of <i>Clausena excavata</i> and Citrus Species	2
1.2.1 General.....	2
1.3 Bioassays of Cytotoxicity, Antimicrobial and Larvicidal Activity...	4
1.3.1 General.....	4
1.4 Objectives of Study.....	6
 2 LITERATURE REVIEW	 7
2.1 Extraction and Isolation of Chemical Constituents from <i>Clausena excavata</i>	7
2.1.1 Previous Works on <i>Clausena excavata</i>	7
2.2 Essential Oils from the Leaves of <i>Clausena excavata</i> and Citrus Species.....	23
2.2.1 Previous Works on Oils of <i>Clausena</i> Species.....	23
2.2.2 Previous Works on Oils of Citrus Species.....	24
2.3 Bioassays of Cytotoxicity, Antimicrobial and Larvicidal Activity....	28
2.3.1 Previous Works on Bioassays of <i>Clausena</i> Species.....	28
2.3.2 Previous Works on Bioassays of Citrus Species.....	30
 3 EXPERIMENTAL	 35
3.1 Extraction and Isolation of Chemical Constituents from <i>Clausena excavata</i>	35
3.1.1 Material and Methods... ..	35

3.1.2	Extraction of <i>Clausena excavata</i> (Stem Bark).....	37
3.1.3	Extraction of <i>Clausena excavata</i> (Leaves).....	49
3.2	Essential Oils from the Leaves of <i>Clausena excavata</i> and Citrus Species.....	52
3.2.1	Plant Materials.....	52
3.2.2	Isolation of the Oils.....	53
3.2.3	GC/MS Analysis.....	55
3.2.4	Identification of the Oil Constituents.....	56
3.3	Bioassays of Cytotoxicity, Antimicrobial and Larvicidal Activity....	56
3.3.1	Cytotoxicity Assay.....	57
3.3.2	Antimicrobial Activity.....	57
3.3.3	Larvicidal Assay.....	59
4	RESULTS AND DISCUSSIONS	61
4.1	Bioactive Principles from the Stem Bark of <i>Clausena excavata</i>	61
4.1.1	Isolation of Scopoletin (8).....	62
4.1.2	Isolation of Stigmasterol (102).....	69
4.1.3	Isolation of Clausine-H (23).....	76
4.1.4	Isolation of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole (103).....	87
4.1.5	Isolation of Clausine-B (21).....	98
4.1.6	Isolation of CEA 10.....	100
4.1.7	Isolation of 1,8-dihydroxy-3-formyl-4-prenylcarbazole (104).....	105
4.1.8	Isolation of Clausine-K (25).....	118
4.1.9	Isolation of Clausenarin (105).....	130
4.2	Bioactive Principles from the Leaves of <i>Clausena excavata</i>	140
4.2.1	Isolation of β -sitosterol (106).....	140
4.2.2	Isolation of Safrole (4).....	142
4.2.3	Isolation of 3-formyl-2,7-dimethoxycarbazole (107).....	151
4.3	Essential Oils from <i>Clausena excavata</i>	163
4.4	Essential Oils from Citrus Species.....	164
4.5	Cytotoxicity Screening.....	181
4.6	Antimicrobial Activity.....	184
4.7	Larvicidal Activity.....	187
5	CONCLUSION	190
	BIBLIOGRAPHY	193
	APPENDICES	199
	VITA	217

LIST OF TABLES

TABLE		Page
1	Summarization of Compounds Isolated from <i>Clausena excavata</i>	20
2	¹ H-NMR Chemical shift Data (ppm) of Scopoletin and Observed Values.....	63
3	¹³ C-NMR Chemical shift Data (ppm) of Scopoletin and Observed Values.....	63
4	¹³ C-NMR Chemical shift Data (ppm) of Stigmasterol and Observed Values.....	71
5	¹ H-NMR Chemical shift Data (ppm) of Clausine-H and Observed Values.....	77
6	¹³ C-NMR Chemical shift Data (ppm) of Clausine-H and Observed Values.....	78
7	¹ H, ¹³ C-NMR Chemical Shifts (δ) and Coupling Patterns of the Protons in HETCOR, COSY and DEPT Techniques of Clausine-H.....	79
8	¹ H-NMR Chemical shift Data (ppm) of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole and Clausine-H.....	88
9	¹³ C-NMR Chemical shift Data (ppm) of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole and Clausine-H.....	89
10	¹ H, ¹³ C-NMR Chemical Shifts (δ) and Coupling Patterns of the Protons in HETCOR, COSY and DEPT Techniques of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole.....	90
11	¹ H-NMR Chemical shift Data (ppm) of Clausine-B and Observed Values.....	99
12	¹³ C-NMR Chemical shift Data (ppm) of Clausine-B and Observed Values.....	100
13	¹ H-NMR Chemical shift Data (ppm) of 1,8-dihydroxy-3-formyl-4-prenylcarbazole and Clausine-D.....	106



14	¹ H, ¹³ C-NMR Chemical Shifts (δ) and Coupling Patterns of the Protons in HETCOR, COSY, DEPT and HMBC Techniques of 1,8-dihydroxy-3-formyl-4-prenylcarbazole.....	108
15	¹ H-NMR Chemical shift Data (ppm) of Clausine-K and Observed Values.....	119
16	¹³ C-NMR Chemical shift Data (ppm) of Clausine-K and Observed Values.....	120
17	¹ H, ¹³ C-NMR Chemical Shifts (δ) and Coupling Patterns of the Protons in HETCOR, COSY, DEPT and HMBC Techniques of Clausine-K.....	121
18	¹³ C-NMR Chemical shift Data (ppm) of Deacetylnomilin, Clausenarin and Observed Values.....	132
19	¹ H, ¹³ C-NMR Chemical Shifts (δ) and Coupling Patterns of the Protons in HETCOR and DEPT Techniques of Clausenarin.....	133
20	GC-MS Analysis of Constituents DH 4.....	147
21	¹ H-NMR Chemical shift Data (ppm) of 3-formyl-2,7-dimethoxycarbazole and Observed Values.....	152
22	¹³ C-NMR Chemical shift Data (ppm) of 3-formyl-2,7-dimethoxycarbazole and Observed Values.....	153
23	¹ H, ¹³ C-NMR Chemical Shifts (δ) and Coupling Patterns of the Protons in HETCOR, COSY and DEPT Techniques of 3-formyl-2,7-dimethoxycarbazole.....	155
24	Composition of the Essential Oil of <i>Clausena excavata</i> (Jabi A, Kedah) Leaves.....	163
25	Composition of the Essential Oil of <i>Clausena excavata</i> (Jabi B, Kedah) Leaves.....	164
26	Composition of the Essential Oil of <i>Citrus microcarpa</i> (Langgar, Kedah) Leaves.....	167
27	Composition of the Essential Oil of <i>Citrus sinensis</i> (Langgar, Kedah) Leaves.....	169
28	Composition of the Essential Oil of <i>Citrus hystrix</i> (Serdang, Selangor) Leaves.....	171
29	Composition of the Essential Oil of <i>Citrus aurantifolia</i> (Serdang, Selangor) Leaves.....	171

30	Composition of the Essential Oil of <i>Citrus maxima</i> (Hutan Kampung, Kedah) Leaves.....	175
31	Composition of the Essential Oil of <i>Citrus maxima</i> (Segamat, Johor) Leaves.....	175
32	Comparison Percentage Composition of the Oils of <i>Clausena</i> <i>excavata</i> and Citrus Species.....	179
33	Cytotoxic activity of crude extracts against CEM-SS cells line (T-lymphoblastic leukemia).....	181
34	Cytotoxic activity of pure compounds against CEM-SS cells line (T-lymphoblastic leukemia).....	182
35	Cytotoxic activity of essential oils against CEM-SS cells line (T-lymphoblastic leukemia).....	183
36	Antimicrobial activity test of crude extracts of <i>Clausena</i> <i>excavata</i>	185
37	Antimicrobial activity test of essential oils of <i>Clausena</i> <i>excavata</i> and Citrus species.....	186
38	Larvicidal activity of crude extracts against larvae of <i>Aedes</i> <i>aegypti</i>	188
39	Larvicidal activity of essential oils against larvae of <i>Aedes</i> <i>aegypti</i>	189

LIST OF FIGURES

FIGURE		Page
1	Apparatus of Dean and Stark.....	54
2	Flow Chart for the Isolation of Essential oils.....	55
3	Scheme for the isolation of the stem bark of <i>Clausena excavata</i>	61
4	Mass Spectrum of Scopoletin (8).....	64
5	IR Spectrum of Scopoletin (8).....	65
6	¹ H-NMR Spectrum of Scopoletin (8) (400 MHz, CDCl ₃).....	66
7	Expanded ¹ H-NMR Spectrum of Scopoletin (8) (400 MHz, CDCl ₃).....	67
8	¹³ C-NMR Spectrum of Scopoletin (8) (100 MHz, CDCl ₃).....	68
9	Mass Spectrum of Stigmasterol (102).....	72
10	IR Spectrum of Stigmasterol (102).....	73
11	¹ H-NMR Spectrum of Stigmasterol (102) (400 MHz, CDCl ₃).....	74
12	¹³ C-NMR Spectrum of Stigmasterol (102) (100 MHz, CDCl ₃).....	75
13	IR Spectrum of Clausine-H (23).....	80
14	Mass Spectrum of Clausine-H (23).....	81
15	¹ H-NMR Spectrum of Clausine-H (23) (400 MHz, Me ₂ CO- <i>d</i> ₆).....	82
16	¹ H- ¹ H COSY Spectrum of Clausine-H (23).....	83
17	¹³ C-NMR Spectrum of Clausine-H (23) (100 MHz, Me ₂ CO- <i>d</i> ₆).....	84
18	DEPT Spectrum of Clausine-H (23).....	85
19	¹ H- ¹³ C HETCOR Spectrum of Clausine-H (23).....	86
20	Mass Spectrum of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole (103).....	91

21	IR Spectrum of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole (103).....	92
22	¹ H-NMR Spectrum of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole (103) (400 MHz, Me ₂ CO- <i>d</i> ₆).....	93
23	¹ H- ¹ H COSY Spectrum of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole (103).....	94
24	DEPT Spectrum of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole (103).....	95
25	¹³ C-NMR Spectrum of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole (103) (100 MHz, Me ₂ CO- <i>d</i> ₆).....	96
26	¹ H- ¹³ C HETCOR Spectrum of 3-carbomethoxy-2-hydroxy-7-methoxycarbazole (103).....	97
27	Mass Spectrum of Clausine-B (21).....	101
28	IR Spectrum of Clausine-B (21).....	102
29	¹ H-NMR Spectrum of Clausine-B (21) (400 MHz, Me ₂ CO- <i>d</i> ₆).....	103
30	¹³ C-NMR Spectrum of Clausine-B (21) (100 MHz, Me ₂ CO- <i>d</i> ₆).....	104
31	Mass Spectrum of 1,8-dihydroxy-3-formyl-4-prenycarbazole (104).....	109
32	IR Spectrum of 1,8-dihydroxy-3-formyl-4-prenycarbazole (104).....	110
33	¹ H-NMR Spectrum of 1,8-dihydroxy-3-formyl-4-prenycarbazole (104) (400 MHz, Me ₂ CO- <i>d</i> ₆).....	111
34	¹ H- ¹ H COSY Spectrum of 1,8-dihydroxy-3-formyl-4-prenycarbazole (104).....	112
35	¹³ C-NMR Spectrum of 1,8-dihydroxy-3-formyl-4-prenycarbazole (104) (100 MHz, Me ₂ CO- <i>d</i> ₆).....	113
36	DEPT Spectrum of 1,8-dihydroxy-3-formyl-4-prenycarbazole (104).....	114
37	¹ H- ¹³ C HETCOR Spectrum of 1,8-dihydroxy-3-formyl-4-prenycarbazole (104).....	115

38	HMBC Spectrum of 1,8-dihydroxy-3-formyl-4-prenylcarbazole (104).....	116
39	¹³ C- ¹ H Coupling Pattern Observed in HMBC Spectra of (104).....	117
40	Fragmentation Patterns of (104).....	117
41	Mass Spectrum of Clausine-K (25).....	122
42	IR Spectrum of Clausine-K (25).....	123
43	¹ H-NMR Spectrum of Clausine-K (25) (400 MHz, DMSO- <i>d</i> ₆).....	124
44	¹ H- ¹ H COSY Spectrum of Clausine-K (25).....	125
45	¹³ C-NMR Spectrum of Clausine-K (25) (100 MHz, DMSO- <i>d</i> ₆).....	126
46	DEPT Spectrum of Clausine-K (25).....	127
47	¹ H- ¹³ C HETCOR Spectrum of Clausine-K (25).....	128
48	HMBC Spectrum of Clausine-K (25).....	129
49	¹³ C- ¹ H Coupling Pattern Observed in HMBC Spectra of (25).....	130
50	IR Spectrum of Clausenarin (105).....	134
51	¹ H-NMR Spectrum of Clausenarin (105) (400 MHz, Me ₂ CO- <i>d</i> ₆).....	135
52	¹ H- ¹ H COSY Spectrum of Clausenarin (105).....	136
53	¹ H- ¹³ C HETCOR Spectrum of Clausenarin (105).....	137
54	¹³ C-NMR Spectrum of Clausenarin (105) (100 MHz, Me ₂ CO- <i>d</i> ₆).....	138
55	DEPT Spectrum of Clausenarin (105).....	139
56	Scheme for the isolation of the leaves of <i>Clausena excavata</i> ...	140
57	IR Spectrum of β-sitosterol (106).....	143
58	Mass Spectrum of β-sitosterol (106).....	144
59	¹ H-NMR Spectrum of β-sitosterol (106) (400 MHz, CDCl ₃)..	145
60	¹³ C-NMR Spectrum of β-sitosterol (106) (100 MHz, CDCl ₃)..	146

61	GC Spectrum of Safrole (4).....	148
62	Mass Spectrum of Safrole (4).....	149
63	IR Spectrym of Safrole (4).....	150
64	Mass Spectrum of 3-formyl-2,7-dimethoxycarbazole (107)....	156
65	IR Spectrum of 3-formyl-2,7-dimethoxycarbazole (107).....	157
66	¹ H-NMR Spectrum of 3-formyl-2,7-dimethoxycarbazole (107) (400 MHz, Me ₂ CO- <i>d</i> ₆).....	158
67	¹ H- ¹ H COSY Spectrum of 3-formyl-2,7-dimethoxycarbazole (107).....	159
68	¹³ C-NMR Spectrum of 3-formyl-2,7-dimethoxycarbazole (107) (100 MHz, Me ₂ CO- <i>d</i> ₆).....	160
69	DEPT Spectrum of 3-formyl-2,7-dimethoxycarbazole (107)...	161
70	¹ H- ¹³ C HETCOR Spectrum of 3-formyl-2,7- dimethoxycarbazole (107).....	162
71	Gas Chromatogram of the Essential oil of <i>Clausena excavata</i> (Jabi A, Kedah).....	165
72	Gas Chromatogram of the Essential oil of <i>Clausena excavata</i> (Jabi B, Kedah).....	166
73	Gas Chromatogram of the Essential oil of <i>Citrus microcarpa</i> .	168
74	Gas Chromatogram of the Essential oil of <i>Citrus sinensis</i>	170
75	Gas Chromatogram of the Essential oil of <i>Citrus hystrix</i>	172
76	Gas Chromatogram of the Essential oil of <i>Citrus aurantifolia</i> .	174
77	Gas Chromatogram of the Essential oil of <i>Citrus maxima</i> (Hutan kampung, Kedah).....	176
78	Gas Chromatogram of the Essential oil of <i>Citrus maxima</i> (Segamat, Johor).....	177

LIST OF ABBREVIATIONS

α	alpha
β	beta
δ	chemical shift in ppm
γ	gamma
μg	micro gram
brs	broad singlet
^{13}C	carbon-13
CHCl_3	chloroform
CDCl_3	deuterated chloroform
COSY-	Correlated Spectroscopy
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarisation Transfer
DMSO	dimethylsulfoxide
dt	doublet of triplet
EA	ethyl acetate
g	gram
GC	Gas Chromatography
GC-MS	Gas Chromatography-mass spectroscopy
^1H	proton
HETCOR	Heteronuclear Chemical Shift-correlation
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
IC	Inhibition Concentration
IR	Infra Red
J	coupling constant in Hz
l	liter
LC	Lethal Concentration
LD	Lethal Dose
m	multiplet
ml	mili liter
mg	mili gram
Me_2CO	acetone
MeOH	methanol
m. p.	melting point
MS	Mass Spectrum/Spectra/Spectrometer/Spectroscopy
NMR	Nuclear Magnetic Resonance
pet. ether	petroleum ether
PLC	Preparative Thin Layer Chromatography
ppm	part per million
s	singlet
t	triplet
TLC	Thin Layer Chromatography
UV	Ultra Violet
WHO	World Health Organization



CHAPTER 1

INTRODUCTION

1.1 Extraction and Isolation of Chemical Constituents from *Clausena excavata*

1.1.1 *Clausena excavata* species

Clausena belongs to the Rutaceae family. It is a genus of about fourteen species of evergreen trees, occurring mostly in India and tropical Asia. *Clausena excavata* is a shrub with strong and rather objectionable smell, found from the Himalayas and China to and throughout Malaysia; particularly in the Peninsula. The local names of this species are “Cherek hitam”, “Chemama” and “Kemantu hitam” (Burkill, 1966).

A slender tree to 10 m tall, its twigs are finely hairy. The leaves are pinnate, 60 cm long with 10-15 pairs of dark green narrowly oval oblique leaflets 3.5-7 cm long and with pointed tips. The leaflets have a characteristic curry-like smell when crushed. Small white flowers occur in terminal clusters, followed by translucent pink berries 7-10 mm across, each containing 1-2 seeds. (Swarbrick, 1997).

Clausena excavata is valued for its medicinal properties. It is also traditionally used in the treatment of snakebite, abdominal pain and as a detoxification agent. A decoction of the roots is drunk for bowel-complaints, chiefly colic. The pounded root is used as a poultice for sores including ulceration of the nose and the leaves are used also for poulticing. It is recorded that pounded they

may be applied to the head for headaches. The flowers and leaves may be boiled and the decoction taken for colic and a decoction of leaves is given after childbirth (Burkill, 1966).

The leaves of this plant are used as a traditional medicine to cure cold, abdominal pain, malaria and dysentery (Wu *et al.*, 1992). The dried and powdered rootstock can be used to treat decayed teeth, whereas its stem is given in colic with or without diarrhea (Kirtikar and Basu, 1933).

1.2 Essential Oils from the Leaves of *Clausena excavata* and Citrus Species

1.2.1 General

Essential oils are the odours of plants due to specific mixtures of volatile substances, which in general are liquid at ordinary temperatures. Essential oils are also called Ethereal or Volatile oils. They occur in small concentrations in special cells, glands or ducts, either in one particular organ of the plant or distributed over many parts e. g. leaves, barks, roots, flowers or fruits (Gupta, 1977).

Occasionally, they are present in combination with sugar as glycosides, *e.g.* amygdalin in bitter almonds and sinigrin in mustard seeds and are liberated when the glycosides are hydrolysed. Essential oils are insoluble in water but freely soluble in alcohol, ether, fatty oils and mineral oils. They are commonly liquid at ordinary temperatures and some of them deposit solid matters on standing. Most of the essential oils are optically active, are lighter than water and possess a high refractive

index. They are composed of a number of chemical compounds – hydrocarbons (terpenes, sesquiterpenes and diterpenes), alcohols, esters, aldehydes, ketons, oxides and lactones, and occasionally compounds of nitrogen and sulphur (Gupta, 1977).

The function of essential oils in the living plant tissue is not completely understood. Odours of flowers for instance may be directly associated with insect attraction or repulsion and it influences pollination and to some extent natural selection. Some are thought to act as a form of protection against parasites and other have such a repulsive odour as to give the plant protection from animal depredation (Lawrence, 1979).

Essential oils may be separated from plant materials by one or the other of following methods (Gupta, 1977):

- i) Distillations (water, water and steam and live steam) are applicable to a wide range of materials.
- ii) Extraction by volatile solvents, hot oils or fats (maceration) or cold neutral fats.
- iii) Expression by hand or machinery, applicable especially to fruit rinds.

Citrus, Linn. A genus of fruit-trees of the family Rutaceae of Asiatic origin and cultivated throughout the warmer parts of the world. It may be stated at once that the aromatic substances in the rind develop best in dry subtropical climates, so that essential oil industries can scarcely be established competitively in the tropics. Different parts of the trees produce volatile oils, right from the seedling stage. These oils are not constant in composition but change in constitution after their first